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Original Article

Lumbar puncture in febrile neonates: a hospital-based cohort study

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Abstract: To provide the evidence of lumbar puncture (LP) related to febrile neonates, we discussed the risk factors of abnormal cerebrospinal fluid (CSF) test for febrile neonates. A retrospective cohort study was conducted on 566 febrile neonates with LP. Clinical characteristics were compared and non-conditional logistic regression analysis was performed to determine independent predictors for LP with abnormal CSF test. There were 146 (25.80%) cases of abnormal CSF test. By logistic regression analysis, the following factors independently predicted abnormal CSF test in febrile neonates: enterovirus infection [odds ratio (OR): 4.652; 95% confidence interval (CI): 2.254-12.248], highest temperature >38.35°C (OR: 2.948; 95% CI: 1.733-5.014), age of onset of fever ≤9.5 days (OR: 2.341; 95% CI: 1.435-3.831), positive blood culture (OR: 2.163; 95% CI: 1.327-3.678), premature rupture of membrane (OR: 2.088; 95% CI: 1.245-3.496), duration of antibiotic use before LP ≤1.5 days (OR: 1.898; 95% CI: 1.278-2.819) and duration of fever lasting >3.5 days (OR: 1.072; 95% CI: 1.021-1.125). The risk factors of abnormal CSF test in febrile neonates were enterovirus infection, highest temperature >38.35°C, age of onset of fever ≤9.5 days, positive blood culture, premature rupture of membrane, duration of antibiotic use before LP ≤1.5 days and duration of fever lasting >3.5 days. Thus, LP should be considered in the high risk infants but not a routine test for all febrile neonates.

Keywords: Lumbar puncture, neonate, febrile, risk factor, cerebrospinal fluid

Introduction

Neonate fever is associated with neonatal sepsis or intracranial infection. Newborns have different anatomy and physiology. The development of neonatal brain is not yet mature, and has an immature blood brain barrier, so intracranial infection in newborns are easy to occur. Since fever may be associated with intracranial infection in newborns, deciding whether to perform lumbar puncture (LP) in newborns may be a challenge; especially because the clinical features of intracranial infection in neonatal infants may be less specific.

There were no unified diagnostic criteria for neonates with intracranial infection. Recent studies showed that the mean cerebrospinal fluid (CSF) white blood cell (WBC) count in the first 28 days of life was 5 and 9.2 per cu.mm, respectively [1-3]. Moreover, Kestenbaum et al [1] showed that the 95th percentile value for CSF WBC in the first 4 weeks of life was 19 WBC per cu.mm. Intracranial infection can lead to various complications and sequelae, pyogenic intracranial infection can lead to subdural effusion, ventricle tube meningitis, syndrome of inappropriate secretion of antidiuretic hormone hydrocephalus, and various nerve dysfunction (such as sensorineural deafness, low intelligence, epilepsy, visual impairment, abnormal behavior) [4-6]. In addition, with the exception of bacterial intracranial infection, viral intracranial infection, such as enterovirus infection or herpes simplex virus infection, is able to cause cerebral edema, cranial pressure, cerebral hernia, associated with a high mortality [7]. Therefore, the early diagnosis and treatment of febrile neonates for intracranial infection is very important.

In order to rule out neonatal bacterial meningitis, all neonatal infants with proven or suspected sepsis should undergo LP [3, 8]. In infants

with fever without an identified source, performing LP to rule out meningitis should be considered in not well-appearing infants and in those ≤21 days old [9]. However, routine LPs are not required for infants (30-90 days) with a fever and a positive urinalysis if they are considered at low risk for serious bacterial infection based on clinical and laboratory criteria [10]. Moreover, although basing the decision to perform LP on proven or suspected sepsis occurs in clinical practice, the diagnostic accuracy of this strategy is unknown. LP is an invasive examination, difficult to operate, can cause tissue damage, headache, herniation, and breathing in cardiac arrest. However, the signs of meningitis are often subtle in the febrile neonates [11]. Therefore, we often perform a LP in neonatal infants with fever, but the evidence of performing LP to febrile neonates is scarce, especially the clinical factors and the necessity of LP are still lacking. Thus, we performed a retrospective cohort study in 566 febrile neonatal infants to compare the clinical characteristics and laboratory test with abnormal CSF test group and the normal CSF test group, and to discuss the risk factors for the abnormal CSF test, and to provide evidence to clinical LP for neonatal infants with fever.

Materials and methods

This study was conducted at the Department of Neonatology of the Children's Hospital, Zhejiang University School of Medicine. The medical chart of 566 febrile neonatal infants, admitted to the department of neonatology between June 2011 and April 2013, were reviewed retrospectively. The institutional research ethics committee approved this study.

Inclusion and exclusion criteria: neonatal infants were considered for study if they met the following criteria: fever (rectal temperature above 38°C). Neonatal infants who met the following condition were excluded: failure to obtain complete demographic data, consent, or adequate clinical samples. The diagnostic criteria of CSF abnormalities was based on the CSF WBC count >20 per cu.mm [1-3] in the first 28 days of life or positive CSF culture and pathogenic bacteria found in CSF. The study population was divided into two clinical groups according to diagnosis: normal CSF test group (420 cases) and abnormal CSF test group (146 cases).

Data on patient demographics, underlying disease, procedures, and medications were collected for analysis. The incidence of LP with abnormal CFS test in febrile neonates was studied by multiple regression analysis of relevant factors: gestational age (GA), birth weight, sex, the age of onset of fever, highest temperature, duration of fever lasting, the nervous system performance, duration of antibiotic use before LP, premature rupture of membrane (PROM), positive blood culture, positive urine culture, enterovirus infection, blood leukocytes, hemoglobin, platelet, C-reactive protein (CRP), blood glucose and lactic acid.

GA was based on gestational age assessment and mother's last menstrual period. Birth weight was measured within the first hour of life. PROM was defined as a rupture of membranes prior to the onset of labor at or beyond 37 weeks of gestation. Nervous system positive performance in this study referred to the conscious state change and mental behavior, bulging fontanelle, muscle strength and muscle tone changes, and positive meningeal stimulation. Enterovirus infection diagnosis was confirmed by a positive enterovirus reverse transcription polymerase chain reaction (RT-PCR) on stool or CSF.

Moreover, we used a cutoff of 38.35°C of highest temperature because it corresponded to the median time of highest temperature in our sample and because temperature >38.35°C was associated with increased likelihood of cerebrospinal fluid abnormalities. We used a cutoff of 1.5 days of duration of antibiotic use before LP because prior antibiotic use lasting ≤1.5 days was associated with increased likelihood of cerebrospinal fluid abnormalities. We had 3.5 days cutoff of duration of fever because duration of fever lasting >3.5 days was associated with increased likelihood of cerebrospinal fluid abnormalities. We used a cutoff of 9.5 days of the age of onset of fever because the age ≤9.5 days was associated with increased likelihood of cerebrospinal fluid abnormalities.

The odds ratio (OR) associated with a given factor was an estimate of the risk for morbidity of LP with abnormal CSF test when the factor was present relative to that when the factor was absent; 95% confidence intervals (95% CI) were used as a measure of the statistical precision of each odds ratio. Adjustment for other

confounding variables was also made by multiple forward stepwise logistic regression analysis. Variables with a *P*-value of <0.1 in the univariate analyses were selected as candidates for a logistic regression analysis. Comparisons of data were made by chi-square test, Fisher exact test or Student's *t*-test. The Mann-Whitney *U*-test was used for variables with a non-normal distribution. A $P<0.05$ was considered statistically significant.

Results

A total of 566 patients were enrolled in the study, of which 146 had LP with abnormal CSF test for a prevalence of 25.80%. CSF of the 146 cases revealed a mean WBC count of 120.25±288.78 cells/mm^3 (range: 2-12400 cells/mm^3) and 24 of the146 abnormal CSF test patients had positive CSF cultures. GA was between 30-42 weeks, and the mean GA was 38.83±1.64 weeks. The median birth weight of LP with abnormal results was 3.30 kg (range: 2.05-4.54 kg). The highest temperature was between 37.40-42.00°C, and the mean highest temperature was 38.70±0.67°C. Patients with LP abnormal CSF test had younger age of onset of fever than the normal CSF test group (median age: 13.00 days, range: 0.30-27.00 days *vs.* median age: 16.00 days, range: 0.50-28.00 days; $P<0.01$). The median duration of antibiotic use before LP in patients with LP abnormal results was 1.00 days (range: 0-13.00 days), and was shorter than the normal CSF test group (median age: 2.00 days, range: 0.00-17.00 days) ($P=0.04$). Patients with LP abnormal CSF test had longer duration of fever than the normal CSF test group (median age: 5.00 days, range: 1.00-21.00 days *vs.* median age: 3.00 days, range: 1.00-12.00 days; $P<0.01$). The pathogens infection consisted of 138 cases of enterovirus infection and 156 cases of bacterial infection (91 cases with positive blood culture and 65 with positive urine culture).

We performed several priori sensitivity analyses to determine the cut off at 38.35°C of highest temperature. The ROC indicated that the area under the curve of 38.35°C of temperature was 0.630 and the Youden's index (Sensitivity + Specificity-1) of 38.35°C was 23.8% >23.2% (38.25°C) >21.5% (38.15°C) >19.5% (38.05°C) >18.4% (38.45°C) >15.6% (38.55°C). Also, we performed several priori sensitivity analyses to determine the cut off at 1.5 days of duration on antibiotic use before LP. The ROC indicated that the area under the curve of 1.5 days was 0.558 and the Youden's index (Sensitivity + Specificity-1) of 1.5 days was 10.6% >9.2% (2.5 days) >8.3% (3.5 days) >2.3% (1.0 day) >0.7% (0.5 day). We performed several priori sensitivity analyses to determine the cut off at 3.5 days of duration of fever lasting. The ROC indicated that the area under the curve of 3.5 days of duration of fever lasting was 0.655 and the Youden's index (Sensitivity + Specificity-1) of 3.5 days was 24% >23.9% (2.5 days) >16.7% (4.5 days) >16.1% (5.5 days) >14.1% (1.5 days). Finally, we performed several priori sensitivity analyses to determine the cut off at 9.5 days of the age of onset of fever. The ROC indicated that the area under the curve of 9.5 days of the age of onset of fever was 0.583 and the Youden's index (Sensitivity + Specificity-1) of 9.5 days was 14.7% >14.6% (8.5 days) =14.6% (7.5 days) >13.5% (10.5 days) >12.9% (6.5 days) >10.9% (11.5 days).

Univariate analysis comparing abnormal CSF test group and normal CSF test group was summarized in **Table 1**. Comparison between two groups demonstrated that there was no association with gestational age, sex, birth weight, nervous system performance, positive urine culture, hemoglobin, platelet, CRP and lactic acid. LP with abnormal CSF test in febrile neonates was correlated with the following factors: highest temperature >38.35°C, duration of fever lasting >3.5 days, age of onset of fever ≤9.5 days, duration of antibiotic use before LP ≤1.5 days, PROM, positive blood culture, enterovirus infection, blood leukocytes and blood glucose. After multivariate analysis, only enterovirus infection, highest temperature >38.35°C, age of onset of fever ≤9.5 days, positive blood culture, PROM, duration of antibiotic use before LP ≤1.5 days and duration of fever lasting >3.5 days remained significant in the forward stepwise logistic regression model (**Table 2**).

Discussion

We performed a hospital-based cohort study of febrile neonates with LP, to measure the risk factors for and outcomes of LP with abnormal CSF test in febrile neonates. To the best of our knowledge, few previous studies of this size,

Table 1. Comparison of lumbar puncture with abnormal cerebrospinal fluid test and with normal cerebrospinal fluid test in febrile neonates

Factors	Abnormal CSF test (n=146)	Normal CSF test (n=420)	t/x^2	*P*
Gestational age (weeks)	39.00 (32.00-42.00)	39.00 (30.00-42.00)		0.47
Birth weight (kg)	3.30 (2.05-4.54)	3.40 (1.47-5.83)		0.39
Sex				
Male	83 (56.85%)	265 (63.10%)	1.785	0.18
Female	63 (43.15%)	155 (36.90%)		
Highest temperatures (°C)				
>38.35	121 (82.88%)	251 (59.76%)	25.695	<0.001
Duration of antibiotic use before LP ≤1.5 days	91 (62.33%)	210 (50.00%)	6.614	0.01
Duration of fever lasting >3.5 days	106 (72.60%)	204 (48.57%)	25.256	<0.001
Age of onset of fever ≤9.5 days	57 (39.04%)	104 (24.76%)	10.853	<0.01
Nervous system performance	54 (36.99%)	118 (28.09%)	4.206	0.12
PROM	13 (8.90%)	19 (4.52%)	3.897	0.04
Positive blood culture	32 (21.92%)	59 (14.05%)	4.973	0.03
Positive urine culture	13 (8.90%)	52 (12.38%)	1.288	0.26
Enterovirus infection	56 (38.36%)	82 (19.52%)	20.840	<0.001
Blood leukocytes (10^9/L)	13.8 (4.0-46.4)	12.7 (3.0-54.7)		0.04
Neutrophils (%)	45.11±18.01	44.24±16.81	0.530	0.59
Hemoglobin (g/L)	151.90±28.15	147.94±28.53	1.451	0.15
Platelet (10^9/L)	359.34±161.18	349.85±134.41	0.639	0.52
CRP (mg/L)	5.0 (1.0-160.0)	5.0 (1.0-160.0)		0.17
Blood glucose (mmol/L)	5.0 (2.3-19.9)	5.1 (0.5-15.9)		0.04
Lactic acid (mmol/L)	2.3 (0.7-26.9)	2.3 (0.8-36.3)		0.35

Abbreviations: LP: lumbar puncture; CSF: cerebrospinal fluid; PROM: premature rupture of membrane; CRP: C-reactive protein. Comparison between two groups demonstrated that there was no association with gestational age, sex, birth weight, nervous system performance, positive urine culture, hemoglobin, platelet, CRP and lactic acid. LP with abnormal CSF test in febrile neonates was correlated with the following factors: highest temperature >38.35°C, duration of fever lasting >3.5 days, age of onset of fever ≤9.5 days, duration of antibiotic use before LP ≤1.5 days, PROM, positive blood culture, enterovirus infection, blood leukocytes and blood glucose.

describing risk factors of febrile neonates with LP in China, have been conducted.

According to recent studies, only few studies assessed the incidence of LP with abnormal CSF test in febrile neonates. Bas et al [12] reported that the incidence of neonatal meningitis was 0.02%-0.72% (including non-fever baby) and in neonatal sepsis, the incidence of meningitis was 25%. We had found that enteroviruses are responsible for 39.22% of febrile neonates during epidemic peak seasons and abnormal CSF test such as CSF pleocytosis could increase the risk of enterovirus infection [13]. It had been also reported that for children less than 3 months with fever, the incidence of enteroviral meningitis was 4.8% [14]. Goldman et al [15] described that children with fever under 90 days, accompanied with pyuria, the incidence of bacterial meningitis was 0%. This study pointed out the risk of bacterial meningitis in infants who present with fever and pyuria, and consider the necessity of LP. The incidence of LP with abnormal CSF test in febrile neonates of 25.80% in our study is higher than that reported by others. This may be probably because the sample characteristics were different. Other studies had described that the research object were not only febrile neonatal infants, but some were less than 3 months of infants. However, in our study, the screening criteria was febrile neonates (include only the neonatal infants), with a variety of abnormal CSF form (the CSF WBC count was 20 per cu. mm, positive CSF culture, find the pathogenic bacteria in CSF). Recent epidemiologic studies showed that the most common pathogens in neonatal meningitis are *Streptococcus agalac-*

Table 2. Binary logistic regression analysis of risk factors for lumbar puncture with abnormal cerebrospinal fluid test in febrile neonates

Factors	β-Coefficient	OR	95% CI	*P*-Value
Enterovirus infection	1.184	4.652	2.254-12.248	<0.001
Highest temperature >38.35°C	1.081	2.948	1.733-5.014	<0.001
Age of onset of fever <9.5 days	0.852	2.341	1.435-3.831	<0.01
Positive blood culture	0.808	2.163	1.327-3.678	<0.01
PROM	0.736	2.088	1.245-3.496	<0.01
Duration of antibiotic use before LP ≤1.5 days	0.641	1.898	1.278-2.819	<0.01
Duration of fever lasting >3.5 days	0.070	1.072	1.021-1.125	<0.01
Blood leukocytes (10^9/L)	0.008	1.008	0.978-1.038	0.62
Blood glucose (mmol/L)	0.089	0.915	0.803-1.043	0.18

Abbreviations: LP: lumbar puncture; OR: odds ratio; 95% CI: 95% confidence interval; PROM: premature rupture of membrane. After binary logistic regression analysis, only enterovirus infection, highest temperature >38.35°C, age of onset of fever ≤9.5 days, positive blood culture, PROM, duration of antibiotic use before LP ≤1.5 days and duration of fever lasting >3.5 days remained significant in the forward stepwise logistic regression model.

tiae and *Escherichia coli*, causing two thirds of all cases [16]. We also found that the positive blood cultures in febrile neonate patients would increase the risk of abnormal CSF test 2.163 fold and LP should be performed in those babies.

Duration of fever lasting, age of onset of fever, and temperature relationships with LP with abnormal CSF test in febrile neonates had not been reported before. Data from our study suggest that those are the risk factors of LP with abnormal CSF test in febrile neonate patients. This seemed biologically plausible because the younger the age of onset of fever, relatively low immune function, and the defect in body's defense system, the longer the duration of fever lasting. In addition, the higher temperature, shows that the greater the chance of infection, the more prone to CSF abnormalities. Data from our research suggest that use antibiotics before LP easily reduced the morbidity of CSF abnormalities in febrile neonate patients. Kanegaye et al [17] also found that the LP performed before parenteral antibiotic therapy could reduce the positive rate of CSF cultures, so as to reduce the incidence of abnormal CSF. In the adult community acquired bacterial meningitis, antibiotics may reduce CSF to cultivate positive [18]. In our study, the morbidity of neonatal intracranial infection is higher with positive blood cultures and enterovirus infection, which finding has not been reported by others. Bas et al [12] reported that in neonatal sepsis, the incidence of meningitis was 25% (did not indicate the presence of fever), but they did not directly report that positive pathogens has the possibility of intracranial infection. The relationship between total blood leukocytes and CSF abnormalities in febrile neonates had no direct reports, but according to relevant reports, in the age range 1 to 36 months with fever, total blood leukocytes was not a predictor of serious bacterial infection, and it had low predictive value [19]. Thus, the blood leukocyte is not described as independent risk factor for abnormal CSF test in febrile neonates indirectly. In our study, the result is consistent with Pulliam PN et al [20]. We also identified that PROM was an independent predictor of abnormal CSF test in febrile neonates. This variable has not been described as a risk factor. As stated by others, PROM had an additive effect on early onset sepsis among very low birth weight infants and neonates [20, 21]. Van Reempts et al [22] also concluded that patients born after very premature and PROM without oligohydramnios had more frequent perinatal infection. Furthermore, blood glucose played a significant role in the initial univariate analysis, but it was not an independent predictor in the logistic regression analysis. Bacterial infection appeared to be affecting blood sugar levels, and the positive blood cultures might be a real risk factor.

In conclusion, we found that abnormal CSF test are responsible for 25.80% in febrile neonates and identified independent predictors of LP with abnormal CSF test, including enterovirus infection, highest temperature >38.35°C, age of onset of fever ≤9.5 days, positive blood culture, PROM, duration of antibiotic use before LP

≤1.5 days and duration of fever lasting >3.5 days. Studies of intervention and appropriate medical management to decrease the incidence of abnormal CSF test are needed in febrile neonates, to provide guidance for clinical LP for neonatal infant with fever. However, the sample size and the small number of events per group, as well as retrospective study design, substantially limit our conclusions.

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Disclosure of conflict of interest

None.

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